The Pharma Innovation Journal 2018; 7(4): 167-168

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(4): 167-168

www.thepharmajournal.com
Received: 23-02-2018
Accepted: 26-03-2018

Kontham Sampath
Department of Animal Genetics and Breeding, C.V.Sc, PVNR Telangana Veterinary University,
Hyderabad, Telangana, India

Vishweshwar Kumar Ganji
FMD research lab, Indian Veterinary Research Institute, Bengaluru, Karnataka, India

Pottabathula Mallesh
Department of Veterinary Parasitology, C.V.Sc, PVNR Telangana Veterinary University, Hyderabad, Telangana, India

Correspondence
Kontham Sampath
Department of Animal Genetics and Breeding, C.V.Sc, PVNR Telangana Veterinary University, Hyderabad, Telangana, India

A direct PCR approach for gene amplification from whole blood sample of sheep

Kontham Sampath, Vishweshwar Kumar Ganji and Pottabathula Mallesh

Abstract
The genomic DNA extraction and PCR amplification of gene fragment from whole blood sample is a cumbersome process. Here in this study we described a direct PCR amplification of gene fragment from the whole blood sample of sheep bypassing the genomic DNA extraction steps. The detailed technique is explained and the results are suggestive of high accuracy in gene amplification. This can be used to cut short the time and is economic for gene amplification from whole blood sample with the same available reagents as for the conventional method.

Keywords: direct PCR, whole blood sample, genomic DNA extraction

Introduction
The world is advancing with implementation of the rapid and cost effective techniques but still there is a need to develop a proper strategy for rapid PCR amplification of genomic DNA. Friedrich Miescher for the first time isolated DNA in 1869 [1]. The blood is the main source for many gene related studies in mammals [2]. Many advancements have been done by using different preservatives for blood preservation and effective DNA isolation [3-6]. Colony PCR was the one technique that is used for direct PCR amplification of gene fragment from bacterial cells [7]. In this study we explored the advantage of this technique for direct PCR amplification without isolating a genomic DNA.

Materials and methods
Blood samples: A total of 10 sheep blood samples are collected from local slaughterhouse, Hyderabad in EDTA containing vacutainer and stored at 4ºC.
Protocol: 1ml of blood sample is centrifuged at 5000rpm for 5min. RBC is lysed by adding RBC lysis buffer (0.32M sucrose, 1mM Tris HCl (pH 7.5) 5mM $MgCl_2$, 1% Triton X-100) and centrifuging at 5000rpm for 5min to remove the lysate till the pellet becomes white. The pellet of WBC is resuspended in 300µl of nuclease free water (NFW) and used as a template for PCR and the reaction is set up as per the standard protocol [8]. The initial denaturation is done at 96ºC for 5min, 30 cycles of PCR at denaturation of 95ºC for 30sec, annealing of 55ºC for 30sec, extension of 72ºC for 1min and final extension of 72ºC for 10 min. The PCR product is loaded on 1% agarose gel, run at 70V and visualized under UV gel doc. The genomic DNA is used as a positive control and no template control (NTC) as negative control.

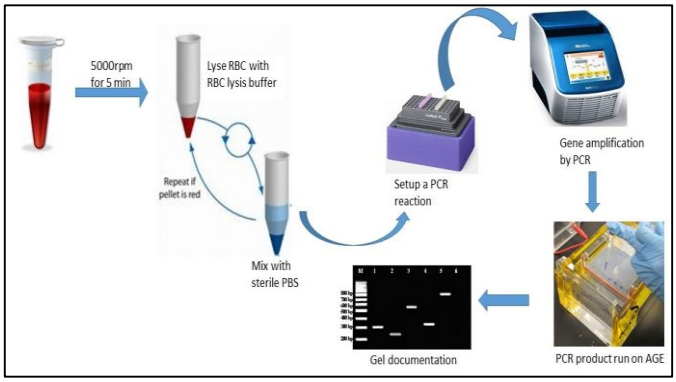

Fig 1: Diagrammatic representation of workflow for Direct PCR amplification of gene fragment from whole blood sample of sheep.

Results

Fig. 2 shows the amplification size of 205 bp in positive samples and a genomic DNA control, no amplification in negative sample and NTC. 9 out of 10 samples shown amplification.

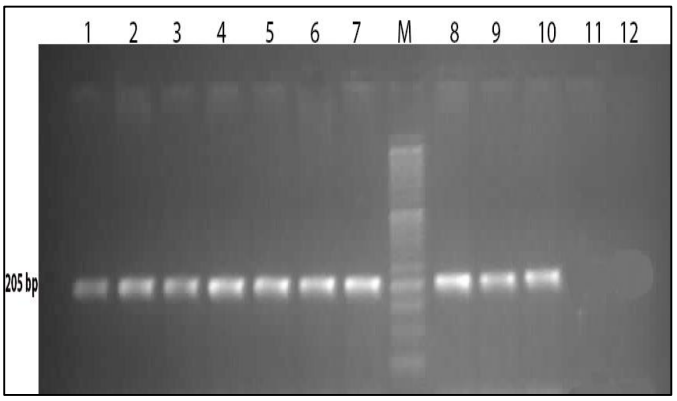

Fig 2: Agarose gel electrophoresis of PCR fragment.

Lane M: Gene Ruler 50bp DNA ladder Lane 1: Genomic DNA positive control show amplification of 205 bp Lane 2-10: WBC cells from samples 1 to 9 respectively show amplification of 205 bp are positive Lane 11: WBC cells from samples 10 show no amplification was negative Lane 12: NTC show no amplification

Discussion

As we mentioned above there is a need for rapid and effective method for PCR amplification of gene from genomic DNA. In this experiment we explored the advantage of using WBC, a kindred of bacterial cells in colony PCR as a template for direct PCR amplification. The results indicate this method is highly specific and effective with high efficiency. The accuracy is high and not a concentration dependant one. The use of direct PCR amplification from whole blood sample saves time and economic. This can be used effectively to bypass the tedious process of genomic DNA isolation. In conclusion we came up with a more rapid, effective and economic way for PCR amplification of gene fragment from genomic DNA of whole blood sample.

References

1. Dahm R. Friedrich Miescher and the discovery of DNA. Developmental biology. 2005; 278(2):274-288.
2. Suguna sajja, Nandal D, Kamble Suresh, Bharatha Ambadasu, Kunkulol Rahul. Genomic DNA isolation from human whole blood samples by nonenzymatic salting out method. Int J pharm pharm sci. 2014; 6:198-199.
3. Albariño CG, Romanowski V. Phenol extraction revisited: a rapid method for the isolation and preservation of human genomic DNA from whole blood. Molecular and cellular probes. 1994; 8(5):423-427.
4. Parzer S, Mannhalter C. A rapid method for the isolation of genomic DNA from citrated whole blood. Biochemical journal. 1991; 273(1):229-231.
5. Subbarayan PR, Sarkar M, Ardalan B. Isolation of genomic DNA from human whole blood. Bio Techniques. 2002; 33(6):1231-1234.
6. Ghatak S, Muthukumaran RB, Nachimuthu SK. A simple method of genomic DNA extraction from human samples for PCR-RFLP analysis. Journal of biomolecular techniques: JBT. 2013; 24(4):224.
7. Bergkessel M, Guthrie C. Colony PCR. Methods Enzymol. 2013; 529:299-309.
8. Mullis KB. U.S. Patent No. 4,683,202. Washington, DC, US. Patent and Trademark Office, 1987.